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L7: Entry 12 of 44

File: USPT

Apr 25, 2000

US-PAT-NO: 6054294

DOCUMENT-IDENTIFIER: US 6054294 A

TITLE: Nucleic acid molecules encoding the neurotrophic factor NNT-1

DATE-ISSUED: April 25, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chang; Ming-shi	Newbury Park	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Amgen Inc.	Thousand Oaks	CA			02

APPL-NO: 08/ 988819 [PALM]

DATE FILED: December 12, 1997

PARENT-CASE:

This application is a divisional of U.S. Ser. No. 08/792,019, filed Feb. 3, 1997, now U.S. Pat. No. 5,741,772, which is incorporated by reference herein.

INT-CL: [07] C12 N 15/11, C12 N 15/63, C12 N 1/21, C07 H 21/04

US-CL-ISSUED: 435/69.1; 435/320.1, 435/325, 435/252.3, 435/254.11, 536/23.51
US-CL-CURRENT: 435/69.1; 435/252.3, 435/254.11, 435/320.1, 435/325, 536/23.51

FIELD-OF-SEARCH: 435/69.1, 435/70.1, 435/71.1, 435/71.2, 435/320.1, 435/325, 435/252.3, 435/254.11, 536/23.1, 536/23.5, 536/23.51, 536/24.3, 536/24.31

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/> 5624806	April 1997	Baker et al.	

OTHER PUBLICATIONS

Boehnnger Mannheim Biochemicals, 1991 catalog, p. 557.
Benigni, et al., Blood 87:5, pp. 1851-1854 (1996).

ART-UNIT: 165

PRIMARY-EXAMINER: Duffy; Patricia A.

ABSTRACT:

Disclosed are nucleic acids encoding novel neurotrophic factors, designated NNT-1. Also disclosed are amino acid sequences for NNT-1 polypeptides, methods for preparing NNT-1 polypeptides, and other related aspects.

9 Claims, 14 Drawing figures

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DOCUMENT-IDENTIFIER: US 6054294 A

TITLE: Nucleic acid molecules encoding the neurotrophic factor NNT-1

YEAR FILED (1):1997Detailed Description Text (38):

The full length NNT-1 polypeptide or fragment thereof can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1989]) and/or Ausubel et al., eds, (Current Protocols in Molecular Biology, Green Publishers Inc. and Wiley and Sons, NY [1994]). A gene or cDNA encoding the NNT-1 protein or fragment thereof may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Alternatively, a gene encoding the NNT-1 polypeptide or fragment may be prepared by chemical synthesis using methods well known to the skilled artisan such as those described by Engels et al. (Angew. Chem. Intl. Ed., 28:716-734 [1989]). These methods include, inter alia, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the NNT-1 polypeptide will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full length NNT-1 polypeptide. Usually, the DNA fragment encoding the amino terminus of the polypeptide will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the NNT-1 polypeptide, depending on whether the polypeptide produced in the host cell is secreted from that cell.

Detailed Description Text (39):

In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants of naturally occurring NNT-1. Nucleic acid variants (wherein one or more nucleotides are designed to differ from the wild-type or naturally occurring NNT-1) may be produced using site directed mutagenesis or PCR amplification where the primer(s) have the desired point mutations (see Sambrook et al., supra, and Ausubel et al., supra, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels et al., supra, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well. Preferred nucleic acid variants are those containing nucleotide substitutions accounting for codon preference in the host cell that is to be used to produce NNT-1. Other preferred variants are those encoding conservative amino acid changes as described above (e.g., wherein the charge or polarity of the naturally occurring amino acid side chain is not altered substantially by substitution with a different amino acid) as compared to wild type, and/or those designed to either generate a novel glycosylation and/or phosphorylation site(s) on NNT-1, or those designed to delete an existing glycosylation and/or phosphorylation site(s) on NNT-1.

Detailed Description Text (40):

The NNT-1 gene or cDNA can be inserted into an appropriate expression vector for expression in a host cell. The vector is selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the NNT-1 gene and/or expression of the gene can occur). The NNT-1 polypeptide or fragment thereof may be amplified/expressed in prokaryotic,

yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend at least in part on whether the NNT-1 polypeptide or fragment thereof is to be glycosylated. If so, yeast, insect, or mammalian host cells are preferable; yeast cells will glycosylate the polypeptide, and insect and mammalian cells can glycosylate and/or phosphorylate the polypeptide as it naturally occurs on the NNT-1 polypeptide (i.e., "native" glycosylation and/or phosphorylation).

Detailed Description Text (46):

The origin of replication element is typically a part of prokaryotic expression vectors purchased commercially, and aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for optimal expression of the NNT-1 polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector.

Detailed Description Text (57):

After the vector has been constructed and an NNT-1 nucleic acid has been inserted into the proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or NNT-1 polypeptide expression.

Detailed Description Text (60):

Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary cells (CHO) or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Detailed Description Text (143):

2. Poly(A).sup.+ RNA Isolation:

Detailed Description Text (144):

Qiagen's Oligotex mRNA isolation system was used as described by the manufacturer; the procedure was repeated twice to obtain pure poly(A).sup.+ RNA. This is especially important for a random primed library to minimize the number of copies of ribosomal RNA in the cDNA. The mRNA integrity was then determined by both spectroscopy and formamide denaturing gel electrophoresis.

Detailed Description Text (147):

Poly(A) RNA was isolated as described above. Approximately 20 mg was then photobiotinylated twice with 20 mg photobiotin acetate (Sigma), and reconstituted at a concentration of 1 mg/ml in RNase-free water. Excess photobiotin was removed with water saturated isobutanol, and ethanol precipitated and resuspended in 30 ml DEPC-treated water.

Detailed Description Text (163):

An expression vector containing human NNT-1 cDNA and flag-tag peptide was constructed by PCR amplification of the fusion gene. A sense primer with Hind III site at the 5' end:

Detailed Description Text (191):

A mouse partial cDNA clone was isolated by PCR amplification from the mouse 11 day-embryo cDNA (Clontech, Palo Alto, Calif.) using the human specific primer. The full-length cDNA clone was further obtained by 5' RACE and 3' RACE. The mouse cDNA nucleotide sequence and amino acid sequence are shown in FIGS. 4 and 5, respectively. The mouse protein shares 96% identity with the human protein, indicating that the protein is highly conserved throughout evolution. Like the human protein, the mouse protein also contains a potential N-linked glycosylation site at amino acid 2 (Asn).

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File: USPT

Nov 17, 1998

US-PAT-NO: 5837493

DOCUMENT-IDENTIFIER: US 5837493 A

TITLE: Human galectins

DATE-ISSUED: November 17, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hillman; Jennifer L.	San Jose	CA		
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Bandman; Olga	Mountain View	CA		
Hawkins; Phillip R.	Mountain View	CA		
Petithory; Joanne R.	Fremont	CA		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Incyte Pharmaceuticals, Inc.	Palo Alto	CA			02

APPL-NO: 08/ 788584 [PALM]

DATE FILED: January 23, 1997

INT-CL: [06] C12 N 15/00, C12 N 1/20, C12 P 21/02, C07 H 21/04

US-CL-ISSUED: 435/69.1; 439/172.3, 439/320.1, 439/252.3, 439/325, 439/348, 439/371, 536/23.1, 536/23.5

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/325, 435/348, 435/371, 536/23.1, 536/23.5

FIELD-OF-SEARCH: 435/320.1, 435/69.1, 435/172.3, 435/252.3, 435/325, 435/348, 435/371, 536/23.1, 536/23.5

PRIOR-ART-DISCLOSED:

OTHER PUBLICATIONS

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ART-UNIT: 162

PRIMARY-EXAMINER: Feisee; Lila

ASSISTANT-EXAMINER: Sun-Hoffman; Lin

ABSTRACT:

The present invention provides two novel human galectins (designated individually as GAL-5HA and GAL-5HB, and collectively as GAL-5H) and polynucleotides which identify and encode GAL-5H. The invention also provides genetically engineered expression vectors and host cells comprising the nucleic acid sequences encoding GAL-5H and a method for producing GAL-5H. The invention also provides for use of GAL-5H and agonists, antibodies, or antagonists specifically binding GAL-5H, in the prevention and treatment of diseases associated with expression of GAL-5H. Additionally, the invention provides for the use of antisense molecules to polynucleotides encoding GAL-5H for the treatment of diseases associated with the expression of GAL-5H. The

invention also provides diagnostic assays which utilize the polynucleotide, or fragments or the complement thereof, and antibodies specifically binding GAL-5H.

9 Claims, 9 Drawing figures

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File: USPT

Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837493 A

TITLE: Human galectins

YEAR FILED (1):1997Detailed Description Text (22):

"Amplification", as used herein, refers to the production of additional copies of a nucleic acid sequence and is generally carried out using polymerase chain reaction (PCR) technologies well known in the art (Dieffenbach, C. W. and G. S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, N.Y.).

Detailed Description Text (25):

The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A". Complementarity between two single-stranded molecules may be "partial", in which only some of the nucleic acids bind, or it may be complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands.

Detailed Description Text (55):

Methods for DNA sequencing which are well known and generally available in the art may be used to practice any embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE.RTM. (US Biochemical Corp, Cleveland, Ohio), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, Ill.), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marketed by Gibco BRL (Gaithersburg, Md.). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, Nev.), Peltier Thermal Cycler (PTC200; MJ Research, Watertown, Mass.) and the ABI 377 DNA sequencers (Perkin Elmer).

Detailed Description Text (58):

Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119). In this method, multiple restriction enzyme digestions and ligations may also be used to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before performing PCR.

Detailed Description Text (83):

The presence of polynucleotide sequences encoding GAL-5H can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or portions or fragments of polynucleotides encoding GAL-5H. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the sequences encoding GAL-5H to detect transformants containing DNA or RNA encoding GAL-5H. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30

nucleotides, and more preferably about 20-25 nucleotides, which can be used as a probe or ampimer.

Detailed Description Text (85):

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding GAL-5H include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding GAL-5H, or any portion thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits from Pharmacia & Upjohn (Kalamazoo, Mich.); Promega (Madison, Wis.); and U.S. Biochemical Corp. (Cleveland, Ohio). Suitable reporter molecules or labels, which may be used, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Detailed Description Text (135):

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding GAL-5H or closely related molecules, may be used to identify nucleic acid sequences which encode GAL-5H. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g., especially in the 3' coding region, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low) will determine whether the probe identifies only naturally occurring sequences encoding GAL-5H, alleles, or related sequences.

Detailed Description Text (140):

In order to provide a basis for the diagnosis of disease associated with expression of GAL-5H, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, which encodes GAL-5H, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with those from an experiment where a known amount of a substantially purified polynucleotide is used. Standard values obtained from normal samples may be compared with values obtained from samples from patients who are symptomatic for disease. Deviation between standard and subject values is used to establish the presence of disease.

Detailed Description Text (156):

The eosinophils used for this library were obtained via apheresis of a 56 year old Caucasian male patient at Mayo Clinic (Rochester Minn.) who had been diagnosed with hypereosinophilic syndrome. The cells were washed twice in phosphate buffered saline and lysed immediately in a buffer containing guanidinium isothiocyanate. The lysate was extracted twice with phenol chloroform and centrifuged over a CsCl cushion using a Beckman SW28 rotor and a Beckman L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated using 0.3M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37.degree. C. The RNA was isolated using the QIAGEN OLIGOTEX kit (QIAGEN Inc, Chatsworth Calif.). Custom cDNA library construction was performed by Stratagene (La Jolla Calif.).

Detailed Description Text (160):

The LNODNOT02 cDNA library was constructed from lymph node tissue removed from a 42 year old, Caucasian female (lot #RA95-05-0297) obtained from the Keystone Skin Bank (International Institute for Advanced Medicine, Exton, Pa.). The tissue was flash frozen, ground in a mortar and pestle, and lysed immediately in a buffer containing guanidinium isothiocyanate. The lysate was extracted twice with acid phenol, pH 4.0, and centrifuged over a CsCl cushion using a Beckman SW28 rotor in a L8-70M Ultracentrifuge (Beckman Instruments). The RNA was precipitated from 0.3M sodium acetate using 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37.degree. C. The poly (A+) RNA was isolated using the QIAGEN OLIGOTEX kit

(QIAGEN Inc.) and used to construct the cDNA library.

Detailed Description Text (180):

By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, PCR is performed using the Peltier Thermal Cycler (PTC200; M. J. Research, Watertown, Mass.) and the following parameters:

Detailed Description Text (196):

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3.times.) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

Other Reference Publication (21):

Jackson, R.J. et al., "Do the poly(A) tail and 3' untranslated region control mRNA translation?", Cell (1990) 62:15-24.